

Cytogenetic Risks and Possible Adverse Health Effects by Narcotic Substances Dependent

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ABSTRACT

Objectives: Illicit drug abuse has crossed social, economic, and geographical borders, and remains one of the major health problems that modern society is facing worldwide. The role of multiple drug abuse as a basic for chromosome damage has been overlooked and it is important to determine its possible adverse health effects. This study aimed to compare the frequency of chromosomal damages between drug addicts and free drug controls.

Methods: Cytogenetic study was obtained from 146 illicit drug-users and 200 free drug controls. Subjects were grouped into three categories depending on main drug of dependence.

Results: Cytogenetic studies on cultured lymphocytes showed an increase the frequency of chromosomal damages among addicts including opiate (5.89%), heroin (7.65%), and crystal (4.9%) when compared with drug free controls (1.45%). The frequency of chromosomal abnormalities was breaks, gaps, marker, and acentric, respectively.

Conclusions: Our findings are also important as they are among the first to suggest here, illicit drug addiction continue to be significant public health problems in Iran.

Key words: Addiction, cytogenetic, health effects, illicit drug, Iran, prevention

INTRODUCTION

Drugs or substances with abuse potential usually possess the property of physical and/or psychological dependence (addiction). Drug abuse not only undermines an individual's/ social health, but also results in major medical expense and induced cancers phenomena.^[1]

Interactions between DNA and environmental factors such as chemical substances are factors known to trigger the process of chromosomal damage and carcinogenesis.^[2] In most studies the role of single and multiple drug abuse as a basic for chromosome damage has been overlooked.^[3-7] It has been estimated that genetic factors contribute to 40%-60% of the vulnerability to drug addiction, and environmental factors provide the reminder.^[8]

For this reason, greater emphasis has been given to methods that detect genotoxic activity in human in an attempt to biomonitor high-risk groups. In view of their association with chromosome aberrations, cytogenetic has been used in studies since 1937 as group indicators of genotoxic exposure.^[3]

Morphine, a parent compound and metabolic of diacetylmorphine (Heroin), initiated many studies to evaluate the cytogenetic damages and health effects of the drug.^[9] There is evidence that *in vivo* administration of morphine to mice can increase the frequency of chromosome aberrations in bone marrow cell^[10] and induce micronuclei in both bone marrow cell and lymphocytes.^[11] Similar cytogenetic results were obtained in studies on diacetylmorphine-treated pregnant rhesus monkey and their offspring.^[12]

Opium is the dried exudates from unripe seed capsules of *Papaver somniferum*. A variety of compounds were later isolated from opium pyrolysates and identified as mutagens. These mutagens were implicated as the cause of opium smoking-induced cancer and chromosomal abnormalities.^[13-15] In Iran, epidemiological studies indicated that opium smoking was associated with esophageal and urinary cancers in human.^[16]

Repeated exposure to numerous drugs of abuse altered gene expression profile throughout the reward circuitry of the (LSD-25) was capable of causing aberrations in human leukocyte chromosomes. Many reports on D-lysergic acid diethylamide (LSD), showing its ability to cause chromosome aberrations *in vitro* and *in vivo*.^[17]

Cannabis is the most widely used illicit drug in the United State of America. Only three sites, one on chromosome 1 and two on chromosome 2 and 9, were identified in the cannabis dependence. A large number of studies have been carried out to associate the mutagenic capacity of cannabis, that is, its ability to react with genicity can be manifested by the induction of structural changes within a chromosomal abnormalities.^[18]

A computer generated bibliography for national and international peer-reviewed publication yielded few reports on drug addicts had introduced into methadone maintenance treatment on opiate dependence in 142 of 230 prisons from Iran.^[16,19] Hence, this is the first cytogenetic research work reported from here

and Middle East on association of chromosome aberration in illicit drug addicts.

METHODS

In January 2008 our laboratory began a study of chromosome damage of one hundred forty six drug abusers admitted to the Loghman major referral hospitals affiliated of Shahid Beheshti University of Medical Sciences (SBUM), Tehran, Iran. Subjects were grouped into three categories depending on main drug of dependence: 101 (69.2%) opiate, 28 (19.2%) heroin, and 17 (11.6%) crystal addicts. The average number of years on drug abuse for all subjects was 12.8 ± 9.4 , but the average for opiate group was 14.2 ± 9.6 years, for the heroin group was 12.0 ± 9.2 years and 5.7 ± 4.1 for crystal users. All drug addicts responded to the check list, applied during an interview. In our studies, informed consent was obtained from all persons. Two hundred free drug control group was matched for age and gender with drug addicts. The volunteers who served as free drug controls were selected from technical staff of Modares, Taleghani and SBMU students. In selecting the free drug controls, we made every effort to involve who had no history of illicit drug abused. The participants did not receive any payment. Only peripheral blood was obtained from each of illicit drug users and free drug controls who were involved in this study.

In this study, 5-7 drops of venous blood was taken from each subject with a heparonized syringe. The blood samples were transferred within a few hours to the laboratory, department of medical genetics, Shahid Beheshti University of Medical Sciences, at culture room temperature (25°C-27°C). Five drops of blood were added to 4 ml of RPMI 1640 (Gibco BRL, USA) medium with 15%-20% FBS (Gibco BRL, USA), L-glutamin (300 µg/ml), 1% penicillin/Streptomycin, and 2% Phytohemagglutinin (Becton Dickinson Co, Ltd USA). The cultures were incubated at 37°C for 72 hr. Colcimide (0.2 µg/ml) (Sigma, Co Germany) was added for 30 minutes, cells were treated with 5 ml of 0.5% KCL for 5 min, and fixed with methanol/acetic acid (Fisher Scientific) (3:1). In most instances, 40 cells from each person were analyzed for possible breaks, dicentric, fragments, rings, exchange fragments, marker, and numerical structure.^[20-23]

The aberration score were: Acentric; a chromatid or chromosome that lacks a centromere. Chromosome

break (br); a visual discontinuity and displacement of both chromatid arms at the same point. Dicentric (dic); a chromosome that is abnormal because it has two centromere. Gaps; a paled or achromatic area of chromatid whose length is not greater than the width of the chromatid. Isochromose (i); a chromosome with identical arms, forming when the centromere splits in the wrong plane. Marker chromosome (mar); an extra abnormal chromosome of unidentified origin. Karyotypes were described according to International System for Chromosome Nomenclature (ISCN).^[20]

Statistical analysis

The results of the investigation were statistically analyzed, by applying One-Way ONOVA, chi square, where the statistical differences yielding $P < 0.05$ were considered significant. Data analysis was performed by SPSS (version 11.5, Inc. USA) software.

RESULTS

One hundred and one opium male users, with the age range from 20 to 77 years, (45.2 ± 13.4) with 49 (48%) affected chromosomal damage; twenty eight heroin males, with the age range from 20 to 70 years, (44.2 ± 15.2) with 18 (66.7) had chromosomal aberration; seventeen crystal male users, with the age range from 20 to 52 years, (33.2 ± 7.9) with 11 (64.7) had metaphase chromosomal abnormalities

and free drug control with the age range from 22 to 71 years, (36.1 ± 14.6) bearing 42 (21%) individuals with chromosomal defects were the subject of this study. The number, types, and percent of aberrations in this study are summarized in the table and figure. All types of chromosome damage as listed in methods were noted. The drug population data represent 350 abnormal metaphases (6%) when including cells containing chromatid gaps 56 (0.9%), breaks 233 (4.0%) and other abnormalities 61 (1.0%). The 7,960 cells scored from the controls' peripheral blood had a total of 116 (1.4%) abnormal metaphases. The significant increase in the frequency of chromosome damage occurred in the lymphocytes from the opiate (5.89%) heroin (7.65%) drug abuse population when compared with normal free drug controls ($P < 0.001$). The mean average of total chromosome profile (aberration) for all affected addicts was 78 (4.5 ± 2.3) and controls 42 (3 ± 2.3) exhibited significant different between the two groups ($P < 0.001$) [Table 1].

The aneuploidy was both hypodiploid and hyperdiploid. The magnitude and range of the chromosome complements in the lymphocyte cells of the subjects did not show appreciable deviations from the normal number of 46 chromosomes. No differences was noted when this series of chromosome diploid was compared to free drug control population. A summary and the results in comparison with the frequency of the cells of drug addicts and normal controls are presented in Table 2.

Table 1: Frequencies of aberration of total chromosome profile for all affected addicts and controls

	N-individual affected	Mean standard deviation	N, range damage Min	Chromosome Max	P value
Opium	49	4.8 ± 2.5	1	15	<0.001
Heroin	18	4.4 ± 1.4	2	7	<0.001
Crystal	11	3 ± 2.4	1	8	<0.001
Control	42	3 ± 1.4	1	7	
Total	118	4 ± 2.2	1	15	

Table 2: Frequencies of persons with detail of chromosome-damaged cells in controls and illicit drug addicts

	N (%) persons	N, cell score	Type and number of chromosome aberrations									N (%)
			br	ga	ace	dic	i	r	t	mar	inv	
Opium addicts	101 (69.2)	4037	181	28	7	5	1	1	-	15	-	238 (5.89)
Heroin addicts	28 (19.2)	1032	42	16	4	4	1	1	-	11	-	79 (7.65)
Crystal addicts	17 (11.6)	673	10	12	1	2	-	-	-	7	-	33 (4.90)
Free drug controls	200 (100)	7960	59	42	5	1	-	1	1	7	-	116 (1.45)

t = Translocation, del = Deletion, ga = Gap, dup = Duplication, i = Isochromosome, inv = Inversion, r = Ring chromosome, mar = Markers chromosome, br = Break, dic = Dicentric

DISCUSSION

There are many investigators to evaluate the cytogenetic effects of drug on human cells from different ethnic groups.^[1,8,18,24] The phenomena of illicit drug dependence poses a major problem in our country. Research-based evidence on chromosomal aberrations and health status on illicit drug addicts from developing and transitional countries as well as Iran is extremely limited.^[19] This findings from our area indicates the following important points, as drug users results is significant increase in the frequency of cells with chromosome damage as compared with that observed in the general population. The frequency of chromosome damages was found more among elderly addicts with long-term period of drug abused.

The first publication attributing to illicit drug LSD to damage human chromosomes was reported in 1967.^[25] A review of 126 persons treated with pure LSD revealed a maximum of 18 (14.3%) with high frequency of chromosome aberrations.^[17] With accumulation of evidence by independent investigations reported the epigenetic landscape of illicit drugs and increase risk of developing malignant neoplastic diseases among narcotic drug users.^[24-28] A rise in Sister Chromatid Exchanged (SCE), a rise in chromosome breakage level, and a decrease in DNA repair ability were found in street heroin users.^[3] Increased chromosome breakage rates were found in those who reported single as well as multiple drug abuse.^[7] However, major health problem associated with high-risk lifestyle observed in non-pregnant illicit opiate users are also observed in pregnant users.^[1] It was found that chromosome aberrations were six to seven times higher in drug-exposed newborns than controls.^[28]

Knowing the chromosome regions that are related to addiction is an important first step. A multiple pooling method with 1,497-SNP microarray identified chromosomal regions that might be concerned in susceptibility to the use of illicit drugs in African – Americans and European – American.^[8] Genetic linkage studies, and population association studies identified chromosomal regions that may contribute to vulnerability to addiction. Association genome scanning can also elucidate chromosomal regions and genes that contain allelic variants that predispose to complex disorders, including substance abuse.

Although family history represents one of the greatest risk factors for drug addiction, the genetic basis for such illness remains poorly understood,^[2] with prominent advances in whole sequencing, the search for genetic variants underlying drug addiction is continuing at an escalating pace, however, genetic factors likely explain ~50% of the risk for addiction.^[8]

Some limitation in this research work should be noted. The spread of infectious disease, such as acquired immunodeficiency syndrome (AIDS), and hepatitis B and C, through needle sharing has been our major concern.

This investigation supports the finding by other workers of an increased frequency of chromosome damage in a population of illicit drug users as compared with chromosomes in free drug controls. The damaged chromosomes we observed of those found in free illicit drug controls could be result of the pharmacology agent/agents or the inability of these cells to repair the damaged chromosomes prior to the induction of mitosis. The only unusual chromosome damage was in two cell of control culture in which two dicentric chromosome were detected. Investigation revealed no evidence of drug ingestion or significant illness in either parent of this control individual.

Despite the prevalence report of illicit drug use in Asia and Pacific.^[29] There is no peer review report of narcotic drug substances has been published in Iran to support our data and findings to draw firm conclusion. Estimate of prevalence of drug use are critical to policy development, planning responses and measuring the coverage of program is required.

The grave implication of this report are not restricted to this limited drug users, themselves-estimated to number thousands in this country alone who may have to face an increased risk of developing malignant neoplastic disease, but invade the realm of social conscience with the prospect of malformation and genetic illness for untold future generations. Clearly, accumulation of evidence by independent investigators for prevention and against the deleterious effects of illicit drug is urgently needed.

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